Experimental Neurology 292 (2017) 1–10

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Saturation of long-term potentiation in the dorsal cochlear nucleus and its pharmacological reversal in an experimental model of tinnitus

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ARTICLE INFO

Article history:
Received 4 November 2016
Received in revised form 9 February 2017
Accepted 14 February 2017
Available online 16 February 2017

Keywords:
Synaptic plasticity
Long-term potentiation
Auditory
Synapse
Central auditory system
Release probability

ABSTRACT

Animal models have demonstrated that tinnitus is a pathology of dysfunctional excitability in the central auditory system, in particular in the dorsal cochlear nucleus (DCN) of the brainstem. We used a murine model and studied whether acoustic over-exposure leading to hearing loss and tinnitus, affects long-term potentiation (LTP) at DCN multisensory synapses. Whole cell and field potential recordings were used to study the effects on release probability and synaptic plasticity, respectively in brainstem slices. Shifts in hearing threshold were quantified by auditory brainstem recordings, and gap-induced prepulse inhibition of the acoustic startle reflex was used as an index for tinnitus. An increased release probability that saturated LTP and thereby induced metaplasticity at DCN multisensory synapses, was observed 4–5 days following acoustic over-exposure. Perfusion of an NMDA receptor antagonist or decreasing extracellular calcium concentration, decreased the release probability and restored LTP following acoustic over-exposure. In vivo administration of magnesium-threonate following acoustic over-exposure restored LTP at DCN multisensory synapses, and reduced gap detection deficits observed four months following acoustic over-exposure. These observations suggest that consequences of noise-induced metaplasticity could underlie the gap detection deficits that follow acoustic over-exposure, and that early therapeutic intervention could target metaplasticity and alleviate tinnitus.

1. Introduction

innitus, the pathological percept of phantom sound, affects 10 to 15% of the adult population worldwide (Dawes et al., 2014; Shargorodsky et al., 2010). Tinnitus has been shown to correlate with aberrant neural activity in the dorsal cochlear nucleus (DCN) (Kaltenbach, 2007), the first relay in the auditory brainstem integrating acoustic and multimodal sensory inputs. Tinnitus is still a poorly understood auditory percept with studies suggesting that altered excitability in the DCN initiates a complex sequence of events relayed to higher levels of the auditory pathway (Brozoski et al., 2002; Ma et al., 2006). For example, acoustic overexposure triggering hearing loss and tinnitus has been shown to enhance DCN somatosensory and vestibular synaptic inputs (Barker et al., 2012; Shore et al., 2008) supporting the idea that tinnitus arises in response to enhanced multisensory synaptic transmission to the DCN (Shore et al., 2008).

Tinnitus has been defined as a pathology of synaptic plasticity in the central auditory pathway (Guitton, 2012; Tzounopoulos, 2008). Synaptic plasticity describes alteration in synaptic strength among connected neurons: this can be either increased, as observed with long-term potentiation (LTP); or decreased, as in long-term depression (LTD) (Bear and Malenka, 1994; Bliss and Collingridge, 1993; Malenka and Bear, 2004). Synaptic plasticity itself is subject to activity-dependent variation as it can be dynamically regulated by prior activity, in a process termed 'metaplasticity' (Abraham, 2008). Aberrant plasticity or metaplasticity has been implicated in the pathophysiology of autism spectrum disorder and fragile X syndrome (Oberman et al., 2016). Recent studies also demonstrated links between chronic pain and metaplasticity promoting excessive amplification of ascending nociceptive transmission to the brain (Li and Baccei, 2016), and between persistent LTP inhibition and memory impairment in Alzheimer's disease (Jang and Chung, 2016).

Whereas the presence of LTP has been demonstrated in the DCN (Tzounopoulos et al., 2004), direct evidence demonstrating metaplasticity in response to acoustic over-exposure triggering tinnitus has yet to be provided. Here we investigate the effect of acoustic over-exposure on plasticity at DCN multisensory synapses and a potential therapeutic reversal of this effect that also ameliorates perception of tinnitus.

2. Materials and methods

One hundred and eight Wistar rats (male and female) were used. Experiments were performed in accordance with the UK Animals

Abbreviations: DCN, dorsal cochlear nucleus; EPSC, excitatory post-synaptic potential; HFS, high frequency stimulations; LTP, long-term potentiation; Mg^{2+}, magnesium; NMDA, *N*-methyl-D-aspartate; PPR, paired pulse ratio; PSFP, post-synaptic field potential.

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http://dx.doi.org/10.1016/j.expneurol.2017.02.011

(Scientific Procedures) Act of 1986 Home Office regulations and approved by the Home Office and Leicester University Ethical Committee (PIL 80/8158, PPL 60/4351).

2.1. Acoustic over-exposure

Rats were aged P15-P18 at the first day of acoustic over-exposure, which corresponds to the period after hearing onset (Geal-Dor et al., 1993). Rats were anesthetised with an intraperitoneal injection of fentanyl (0.15 mg/kg), fluanisone (5 mg/kg, VetaPharma Ltd) and Hypnovel (2.5 mg/kg, Roche). Using this combination of anaesthetics, rats were initially anesthetised for about 1 h, after which animals stayed sedated. Rats were placed in a custom made open field sound-insulated chamber containing a 600 W High Power Horn Tweeter radiating evenly, frequency range 2–20 kHz (Maplin UK) so that both ears were exposed. Bilateral noise exposure was used as it best approximates the noise exposure that occurs in humans (Metidieri et al., 2013). A pure tone of 14.8 kHz was delivered at 110 dB SPL for a total of 9 h (3 h per day over 3 consecutive days) as previously described (Tagoe et al., 2014). Age-matched control animals from the same litter were similarly anesthetized but unexposed to acoustic over-exposure. In vitro auditory brainstem recordings or gap detection screening following the acoustic over-exposure or the anaesthesia only were performed blind.

2.2. Auditory brainstem response recordings

Rats were anesthetised using similar anaesthetics as mentioned above. Auditory brainstem response recordings were performed at three time points: before, 4 days, and 18 weeks after anaesthesia only (controls) or after acoustic over-exposure. Positive, negative, and ground electrodes were inserted subcutaneously at the vertex, mastoid, and back, respectively (Pilati et al., 2012b). Auditory brainstem responses were evoked by calibrated tone pips (8, 16, 24, 30 kHz; 1 ms rise and fall times, 5 ms duration, 3 ms plateau) generated in a free field at 10 Hz by a waveform generator (TGA 1230 30 MHz, Tucker Davis Technology, USA) and an acoustic driver (Bruel & Kjaer type 4192, Denmark). Evoked responses were recorded by an amplifier (Medelec Sapphire 2A, Oxford Instruments, UK), band-pass filtered between 10 Hz and 5 kHz and averaged from 300 to 400 Hz sweeps or 800 to 1000 sweeps at threshold using a custom made software (CAP, GSK). Tone pips were progressively attenuated in 10 or 3 dB SPL steps from an initial intensity of 94 dB SPL using a digital attenuator (PA4, Tucker Davis Technology, USA). Hearing thresholds were defined as the lowest sound pressure level at which peaks 1 and 2 could be recognized (Barker et al., 2012; Pilati et al., 2012a; Tagoe et al., 2014). Detection of peaks was confirmed by comparing the auditory brainstem waveform with two or three suprathreshold waveforms. Final determination of threshold was made by reanalysing the traces off-line. Threshold shifts were used as the primary indicator of hearing performance and were measured at the left ear as the difference between the hearing threshold on day 1 (P15–18) and the hearing threshold 4 days after the acoustic over-exposure procedure.

2.3. Behavioural assessment of tinnitus

The behavioural assessment of tinnitus is based on the gap detection paradigm originally described by (Turner et al., 2006). The paradigm is based on the pre-pulse inhibition of the acoustic startle reflex whereby the startle reflex is inhibited by a short silent gap embedded in a continuous background noise. Turner et al. (2006) demonstrated selective gap detection deficits in rats following acoustic over-exposure that they hypothesised were due to tinnitus. Gap detection deficits were assessed using a specific acoustic startle reflex hardware and software (Kinder Scientific, Poway, CA). Each rat was presented with a constant 65 dB SPL background noise consisting of octave based sounds centred at either 8 kHz, 16 kHz, 24 kHz, 30 kHz or broadband noise (BBN). A 110 dB SPL, 20 ms BBN noise burst served as the startle stimulus to induce the acoustic startle reflex. During the background noise, the rat was either presented with the startle stimulus alone (startle only condition) or the startle stimulus preceded by a silent gap embedded within the background noise (GAP condition). Silent gaps (50 ms in duration with a 0.1-ms rise/fall) began 100 ms before the startle stimulus. Each testing session began with a 2-minute acclimatisation period to the background sound. This was followed by two trials of startle stimuli to trigger initial startle reflexes that were excluded from the analysis. The testing phase consisted of mixing a pseudo-random sequence of 12 startle only trials (with no silent gaps) with 12 trials containing a silent gap, both embedded in similar background noise preceding the startle stimulus. Startle responses were converted into gap detection ratios (GDRs) whereby for a given frequency, the mean startle response to the gap condition was divided by the mean startle only response. Screening was first performed at P15-P18 where startle response amplitudes were compared in the presence and absence of gaps embedded in broadband noise. This allowed selecting rats displaying an ability to detect gaps prior to the original testing phase. Selected rats were then randomly assigned to either a control or an exposed group and screening was repeated 18 weeks following acoustic over-exposure or anaesthesia only. Auditory brainstem response recordings were used to confirm recovery from hearing loss 18 weeks following acoustic over-exposure, ensuring that the effects on gap detection deficits were specific rather than due to hearing loss.

2.4. Magnesium administration

Magnesium was administered by supplementing normal drinking water with Mg^{2+}-threonate (604 mg/kg/day corresponding to 50 mg/kg/day elemental Mg^{2+} (Abumaria et al., 2011)) on the last day of the acoustic over-exposure for a maximal period of 18 weeks. The dose has previously been shown to be effective in elevating brain Mg^{2+} (Slutsky et al., 2010).

2.5. Multisensory input stimulation

Multisensory inputs to the DCN were stimulated by placing a bipolar stimulating electrode (FHC Inc., USA) in the molecular layer (Oertel and Young, 2004). Field potential and whole cell recordings were performed in the dorsal segment of the fusiform cell layer encoding high frequencies (Muniak and Ryugo, 2014) as previously described (Tagoe et al., 2014).

2.6. Field potential recordings

Our study took advantage of field potential recordings to allow stable and prolonged recordings from a large number of undialysed cells in the DCN fusiform cell layer, including fusiform, granule and cartwheel cells (Oertel and Young, 2004). Using field potentials also limited the risk of washing out intracellular substances that could be essential for studying LTP and metaplasticity (Abrahamsson et al., 2016). This proved beneficial, as we were able to record LTP for at least 60 min and perform various experimental procedures within that time-window. Coronal brainstem slices (250 μm) containing the DCN were obtained from rats 4–5 days after acoustic over-exposure (or anaesthesia only) between P19 and P23, and also 1 month after acoustic over-exposure between P49 and P54. Dissection of the brainstem and slicing procedures were performed as previously described (Barker et al., 2012; Pilati et al., 2012a). Field potential recordings were performed in normal extracellular solution containing (in mM): NaCl 125, KCl 2.5, NaH_2PO4 1.2, D-glucose 10, ascorbic acid 0.5, Na pyruvate 2, myo-inositol 3, $NaHCO_3$ 26, $CaCl_2$ 2 and $MgCl_2$ 0.1. Parallel fiber evoked field potentials recorded in the DCN fusiform layer is a composite of events with nomenclature which has been described previously (Manis, 1989). The amplitude of the N1 or the PSFP (N2) wave was